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(71) Applicant *(for all designated States except US)*: UNIVERSITY OF STRATHCLYDE [GB/GB]; McCance Building, 16 Richmond Street, Glasgow G1 1XQ (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: HABTEMARIAM, Solomon [ET/GB]; University of Greenwich, School of Chemical & Life Sciences, Wellington Street, Woolwich, London SE18 6PF (GB)..

(74) Agents: McCALLUM, William, Potter et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).

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(54) Title: INTEGRIN DEPENDENT CELL ADHESION INHIBITORS

(57) Abstract

The present invention relates to euparin derivatives having broad spectrum integrin receptor inhibition activity and utility in the treatment of integrin mediated disease.

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INTEGRIN DEPENDENT CELL ADHESION INHIBITORS

Background

The present invention relates to integrin dependent cell adhesion inhibitors and more particularly to inhibitors suitable for use in the prophylaxis and treatment of integrin-mediated conditions.

Integrins are a family of cell adhesion molecules that mediate cell-cell and cell matrix interactions. There is now considerable evidence that suggests that integrins play a major role in the pathogenesis of various diseases including angina, arthritis and other inflammatory diseases, asthma, cancer metastasis, coronary angioplasy, psoriasis, osteoporosis, thrombosis and viral and parasitic infections. Other inflammatory conditions to which the present invention relates, that may be specifically mentioned include: Atherosclerosis; Vasculitis; Ischemia and reperfusion injury; Adult respiratory distress syndrome; Renal disease comprising various forms of nephritis; Gastrointestinal inflammation (Inflammatory bowel disease)- Ulcerative colitis and Crohn's disease; Hepatic disease and in particular hepatitis; CNS disease including Multiple sclerosis and encephalitis; Dermatoses; Graft rejection; Graft versus Host disease; and Sepsis. In more detail Sepsis and septic shock occur when the usual inflammatory responses mounted by the body against invading organisms becomes uncontrolled. Around half of septic patients die from the disease and its complications in intensive care units, where septic shock is the most common non-coronary cause of death. Current treatment aims to eradicate the underlying infection and to control the main symptoms using intravenous fluids to maintain vascular volume; vasopressor and/or inotropic drugs if hypotension is still present; diuretics for oliguria; and anticoagulants for disseminated intravascular coagulopathy.

While the discovery of integrin receptors has provided a

useful therapeutic target for the above diseases, the search for small molecular weight antagonists and even the multiplicity (over 22 integrins discovered and many sharing similar functions) of integrin receptors have proved to be challenging - not least in relation to the development of practically useful inhibitors whose effects are not readily negated by integrin receptor redundancy. A relatively non-selective integrin antagonist is the ultimate goal of integrin-based therapy.

Summary of Invention

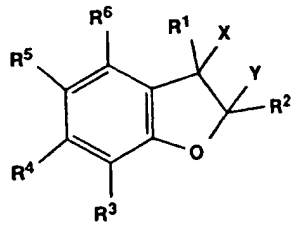
It is an object of the present invention to avoid or minimize one or more of the above problems or disadvantages. It is a further object of the present invention to provide new materials and/or methods for the treatment of one or more of the above mentioned integrin-mediated conditions.

Statement of Invention

As a result of our research we have successfully isolated an integrin antagonist from gravel root (*Eupatorium purpureum*) which we have identified as 3α-tiglinoyl-2,3-dihydroeuparin (5-acetyl-2,3-dihydro-cis-6-hydroxy-2-isopropenyl-3-tiglinoyloxy-benzofuran) which has broad-spectrum integrin receptor inhibition activity.

The present invention provides in a first aspect, use of a compound of general formula (I)

(I)



wherein each one of R^1 and R^2 is independently selected from optionally substituted and/or unsaturated C_1-C_8, preferably C_1-C_6, alkyl and alkoxy, OH, and aryl, or represents ZCO_2 wherein

Z is independently selected in each case from optionally substituted and/or unsaturated C_1-C_8, preferably C_1-C_6, alkyl, and aryl, or

R^1 has the same meaning as before, and R^2 is ZCO_2W wherein Z has the same meaning as before and W is CH_2CO or optionally substituted and/or unsaturated C_1-C_8, preferably C_1-C_6, alkyl;

each one of R^3, R^4, R^5 and R^6 is independently selected from H, OH, Cl, Br, F, I, C_1-C_8, preferably C_1-C_6, alkoxy, C_1-C_8, preferably C_1-C_6, alkyl, R^7CO, NO_2, and NR^8R^9;

R^7 being H or C_1-C_8, preferably C_1-C_6, alkyl, and

each one of R^8 and R^9 being independently selected from H and C_1-C_4 alkyl;

with preferably at least one of R^1 to R^6, most preferably at least one of R^1 and R^2, comprising a ZCO_2 moiety wherein Z has the same meaning as before; and

each one of X and Y is H, one of X and Y is H and the other is OH, or X and Y together represent a double bond,

for the preparation of a medicament for the treatment of an integrin mediated condition.

In a further aspect the present invention provides a pharmaceutical formulation comprising a compound of formula I as defined herein before, or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier therefor.

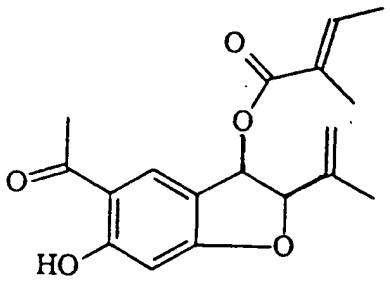
Unless otherwise indicated, alkyl groups comprising R^1 to R^9 or part thereof, in formula (I), may be straight or branched chain alkyl groups such as isopropyl, propyl, butyl, isobutyl, tertbutyl and the like, or cylic, including polycyclic, for example cyclohexyl.

Preferred compounds of general formula I are those in which R^1 represents ZCO_2 wherein Z is optionally unsaturated C_1-C_6 alkyl, R^2 is selected from optionally substituted and/or unsaturated C_1-C_6 alkyl and aryl, and each one of R^3, R^4, R^5 and R^6 is independently selected from H, OH, Cl, Br, F, I, C_1-C_6

alkoxy, C_1-C_6 alkyl, R^7CO, NO_2 and NR^8R^9; R^7 being H or C_1-C_6 alkyl, and each one of R^8 and R^9 being independently selected from H and C_1-C_4 alkyl, and each one of X and Y is H, or X and Y together represent a double bond. Advantageously R^2 represents optionally unsaturated C_1-C_4 alkyl. Desirably R^5 represents CH_3CO. Most desirably R^4 represents OH and each of R^3 and R^6 represents H. Preferably R^1 is tiglinoyl ($CH_3CHC(CH_3)CO_2$).

Particularly preferred compounds of the invention include:

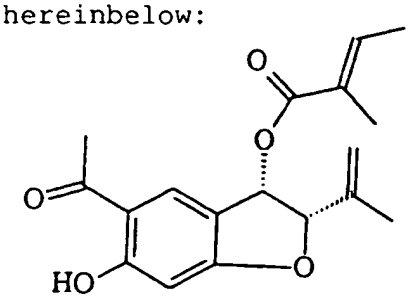
(1)



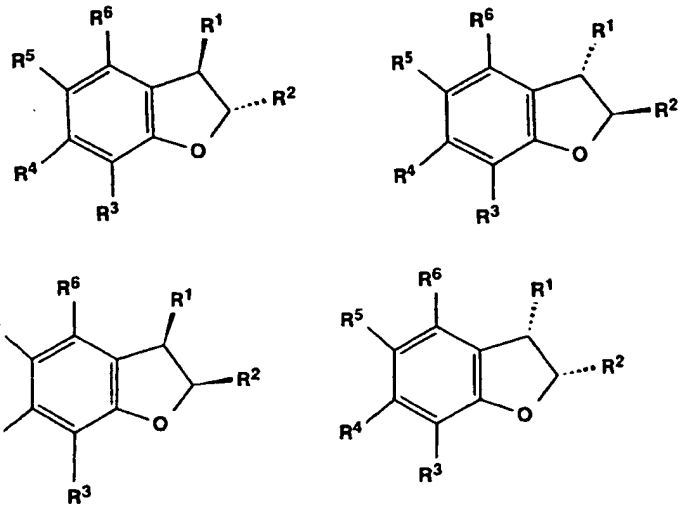
and physiologically functional derivatives thereof.

In yet another aspect the present invention provides as novel compounds, compounds of the general formula I as defined hereinbefore except that when R^1 is $CH_3C:CHCH_3CO_2$, R^2 is $CH_3C:CH_2$, R^4 is OH, and R^5 is $C:OCH_3$, then R^3 and R^6 are not both H.

It will be appreciated that the compounds of general formula I have two optically active centres at the 2 and 3 positions, in the furyl ring, whereby the compounds of formula I include various different stereoisomeric forms. It is believed that the naturally occurring stereoisomer of the preferred compound (1) is the cis form shown hereinbelow:



The skilled addressee will appreciate though that compound 1 may also be obtained in another cis-form and in two different trans-forms. Thus the present invention encompasses the stereoisomeric forms of such compounds of formula I wherein X and Y are both H, as shown hereinbelow:



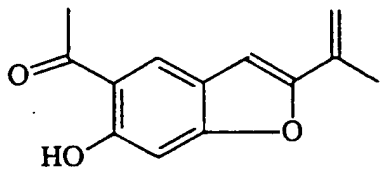
The skilled addressee will appreciate that in the case of compounds of general formula I which contain one or more nitrogen atoms, the present invention encompasses physiologically acceptable acid addition salts thereof, and in the case of compounds of general formula I which contain acidic groups, the present invention encompasses physiologically acceptable salts thereof with bases.

Physiologically functional derivatives for the purposes of the present invention means those derivatives which have useful activity in integrin mediated conditions and especially which have broad-spectrum integrin receptor inhibition activity.

Compounds of general formula I may be prepared by various processes known in the art and the present invention encompasses the use of such processes for the synthesis of novel compounds of the present invention. In the literature various procedures are described for the synthesis of various substituted benzofurans and 2,3-dihydrobenzofurans. (Clavel,

J-M. et al (1977) J. Heterocyclic Chem. 14, 219-224; Yamaguchi, S. et al (1987) Bull. Chem. Soc. Jpn. 60, 3603-3605; Elix, J.A. (1971) Aust. J. Chem. 24, 93-97.) Compounds such as compound 2: euparin

(2)



are also readily obtainable in large quantities by extraction from *Eupatorium* species plant matter, and in particular from *Eupatorium purpureum* (gravel root) rhizome, and can be used as a starting material for the synthesis of various compounds of general formula (I).

As noted hereinbefore (and described in more detail hereinbelow), compound 1 is obtainable from natural sources including *inter alia Eupatorium purpureum* and from *Isocarpha oppositifolia* and the present invention accordingly also encompasses:

Use of compound 1 when isolated and/or purified from natural sources as described hereinbefore, in the preparation of a medicament for the treatment of an integrin mediated condition;

Pharmaceutical formulations comprising compound 1 when isolated and/or purified from natural sources as described hereinbefore, or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier therefor; and

Methods of treatment of an integrin-mediated condition or prophylaxis of an integrin mediated condition, which method comprises the administration of a clinically useful amount of compound 1 when isolated and/or purified from natural sources as described hereinbefore, or a pharmaceutically acceptable salt or physiologically functional derivative thereof in a pharmaceutically useful form, one or more times a day or in

any other appropriate schedule, orally, topically, rectally, or parenterally.

The compounds of the present invention are indicated as being useful in the treatment and prophylaxis of integrin mediated conditions, including those specifically recited hereinbefore, and especially inflammatory conditions. In relation to the latter, the compounds of the present invention offer particular advantages in that they avoid the undesirable side effects, such as gastric irritation, of conventional non-steroidal anti-inflammatory medicaments such as aspirin which function as cyclo-oxygenase inhibitors. The non-steroidal nature of the compounds of the present invention also allows the treatment of conditions such as sepsis and septic shock which cannot be treated with conventional anti-inflammatory medicaments, as well as being more suitable for use in the treatment of chronic inflammatory conditions.

The invention thus further provides a method for the treatment or prophylaxis of an integrin mediated condition, which method comprises the administration of a clinically useful amount of a compound of Formula (I) or a pharmaceutically acceptable salt or physiologically functional derivative thereof in a pharmaceutically useful form, one or more times a day or in any other appropriate schedule, orally, topically, rectally, or parenterally.

The amount of compound of Formula (I) required to be effective in the treatment of an integrin mediated condition will, of course, vary and is ultimately at the discretion of the medical or veterinary practitioner. The factors to be considered include the particular condition being treated, the route of administration, and nature of the formulation, the mammal's body weight, surface area, age and general condition, and the particular compound to be administered. A suitable effective dose generally lies in the range of about 0.01 to

about 120mg/kg bodyweight, e.g. 0.1 to about 120 mg/kg bodyweight, preferably in the range of about 0.1 to 100mg/kg bodyweight. The total daily dose may be given as a single dose, multiple doses, e.g., two to six times per day or by intravenous infusion for a desired duration. For example, for a 75 kg mammal (e.g. a human) the dose range would be about 10 to 1000 mg per day, and a typical dose could be about 50 mg per day.

Whilst it is in principle possible for the active compound to be administered alone, it is preferable to present the active compound in a pharmaceutical formulation. Formulations of the present invention, for medical use, comprise a compound of Formula (I) or a pharmaceutically acceptable salt thereof together with one or more pharmaceutically acceptable carriers and optionally other therapeutic ingredients. The carrier(s) should be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The present invention, therefore, further provides a pharmaceutical formulation comprising a compound of Formula (I) or a pharmaceutically acceptable salt or physiologically functional derivative or precursor thereof together with a pharmaceutically acceptable carrier therefor.

There is also provide a method for the preparation of a pharmaceutical formulation comprising bring into association a compound of Formula (I) or a pharmaceutically acceptable salt or physiologically functional derivative or thereof, and a pharmaceutically acceptable carrier therefor.

Formulations according to the present invention include those suitable for oral, topical (including pulmonary), rectal or parental (including subcutaneous, intramuscular and intravenous administration. Preferred formulations are those

suitable for oral, topical or parenteral administration.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredient. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier or a finely divided solid carrier or both and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active compound; as a powder or granules; or a solution or suspension in an aqueous or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable matching a mixture of the powdered active compound with any suitable carrier.

A syrup may be made adding the active compound to a concentrated, aqueous solution of a sugar, for example sucrose, to which may also be added any accessory ingredients. Such accessory ingredient(s) may include flavorings, an agent to retard crystallization of the sugar or an agent to increase the solubility of any other ingredients, such as a polyhydric alcohol for example glycerol or sorbitol.

Formulations for rectal administration may be presented as a suppository with a conventional carrier such as cocoa butter.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound which is preferably isotonic with the blood of the recipient. Such formulations suitably comprise a solution of a pharmaceutically and pharmalogically acceptable salt of a compound of Formula (I) that is isotonic with the blood of the recipient.

Useful formulations also comprise concentrated solutions or solids containing the compound of Formula (I) which upon dilution with an appropriate solvent give a solution for parenteral administration as above.

For pulmonary administration, the combination is suitably inhaled from a nebulizer, from a pressurized metered dose inhaler or as a dry powder from a dry powder inhaler or from a dry powder inhaler utilizing gelatin, plastic or other capsules, cartridges or blister packs. A diluent or carrier, generally non-toxic and chemically inert to the medicament e.g. lactose, dextrin, mannitol or glucose or any additives can be added to the powdered medicament. The agglomerated, free-flowing micronized mixture may be filled into a dry powder inhaler. When a capsule system is used, it is desirable to include a filler in the mixture.

The micronized mixture may be suspended or dissolved in a liquid propellant mixture which is kept in a container that is scaled with a metering valve and fitted into a plastic actuator. The propellants used may be chlorofluorocarbons of different chemical formulae.

In addition to the aforementioned ingredients, formulations of this invention may further include one or more accessory

ingredient(s) selected from diluents, buffers, flavoring agents, binders, surfactants, thickeners, lubricants, preservatives (including antioxidants) and the like.

Preferred formulations suitable for topical administration, especially to the skin are generally applied as a topical ointment or cream containing the active ingredient in an amount of, for example, 0.075 to 20% w/w, preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glyercol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulphoxide and related analogues.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called

emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glycerol mono-stearate and sodium lauryl sulphate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or di-basic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Other topical formulations suitable for use especially on the skin or mucosa (including rectal, vaginal, nasal or oral mucosa) generally comprise the active ingredient in intimate admixture with a pharmaceutically acceptable vehicle or carrier so as to provide lotions, suspensions, ointments, creams, gels, tinctures, sprays, powders, pastes, slow-release transdermal patches, suppositories, or mouthwashes. The active compounds can also be applied in a time release formulation via patches or in slow release polymers. The active compounds can be prepared with carriers that will

protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems.

In addition to the other materials listed above for systemic administration, thickening agents, emollients, and stabilizers can be used to prepare topical compositions. Example of thickening agents include petroleum, beeswax, xanthan gum, or polyethylene glycol, humectants such as sorbitol, emollients such as mineral oil, lanolin and its derivatives, or squalene. Natural or artificial flavorings or sweeteners can be added to enhance the taste of topical preparations applied for local effect to mucosal surfaces. Inert dyes and colors can also be added.

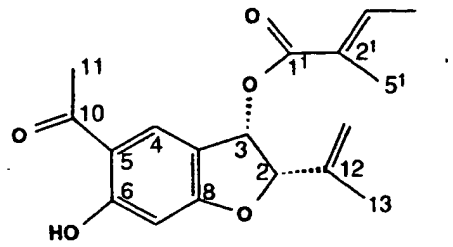
Further preferred features and advantages of the invention will appear from the following illustrative examples.

Example 1- Isolation of 5-acetyl-2,3-dihydro-cis-6-hydroxy-2-isopropenyl-3-tiglinoyloxy-benzofuran

Dried powdered rhizome (Batch number: 01473) of cultivated *Eupatorium purpureum* (gravel root) were supplied by the Herbal Apothecary (Syston, Leicester, UK). 1 kg of the powdered plant material was soaked in 5 l of absolute ethanol for two weeks. The extract was then filtered and taken to dryness under a reduced pressure and then freeze-dried to yield the crude extract (120 g). The crude extract (100g) was then suspended in water (1 L) and successively re-extracted (3-times) with 1 L each of chloroform (yield: 40g), ethyl acetate (6g) and butanol (14g). All fractions including the final remaining water fraction (yield: 35g) were concentrated under reduced pressure using a rotary evaporator and then freeze dried. The chloroform fraction (which had most of the activity) was chromatographed on a silica gel column (3x50 cm) and eluted with 500 ml each of hexane, hexane-$CHCl_3$ mixtures (9:1. 8:2, 6:4, 1:1) of increasing polarity and finally $CHCl_3$. The

hexane-$CHCl_3$ (8:2) eluant was collected and subjected to repetitive preparative TLC (silica gel, Hexane:$CHCl_3$, 1:1) to give compound 1 (40 mg).

(1)



Compound 1 gave UV, IR, EIMS and ^1H NMR (Table 1) data in close agreement with that reported for 5-acetyl-2,3-dihydro-cis-6-hydroxy-2-isopropenyl-3-tiglinoyloxy-benzofuran from *Isocarpha oppositifolia* (Bohlmann, F., Mahanta, P.K., Natu, A.A., King, R.M. and Robinson, H. (1978) Phytochemistry 17, 471-474.). The structure of compound 1 was further confirmed by means of ^1H-^1H COSY, NOESY, ^{13}C NMR and HMBC studies. The ^{13}C-NMR chemical shift data could be assigned unambiguously through ^2J and ^3J HMBC correlation studies and reported here for the first time (Table 1).

Table 1. ^{1}H and ^{13}C chemical shift data and ^{2}J and ^{3}J HMBC correlations for compound **1** (400 MHz, $CDCl_3$).

Atom	^{1}H (J)	^{13}C	^{2}J and ^{3}J correlations
2	5.14 d (6)	89.3	C-13, C-14
3	6.28 d (6)	72.4	C-8, C-9, C-1'
4	7.86 s	130.6	C-3, C-6, C-8, C-10
5		114.9	
6		167.1	
7	6.45 s	99.9	C-5, C-8, C-9
8		166.8	
9		118.4	
10		202.8	
11	2.56 s	26.6	C-10
12		138.3	
13	1.80 s	19.4	C-2, C-12, C-14
14	5.11 br s and 5.23 br s	114.3	C-2, C-12, C-13
1'		167.6	
2'		128.3	
3'	6.83 qq (7, 1)	138.6	C-1', C-4', C-5'
4'	1.78 dq (7, 1)	14.7	C-2', C-3'
5'	1.79 br s	12.1	C-1', C-2', C-3'
OH	13.02 s		C-5, C-6, C-7

Example 2 - Pharmacology

Compounds of the invention were assayed for biological activity as described hereinbelow.

Example 2A - In vivo Anti-inflammatory Activity of Compound 1

Carrageenan oedema test: Male Sprague-Dawley rats weighing between 260-290g were randomly divided into groups of 6-8 animals each. They were maintained on CRM pelleted diet (B. S. & S. (Scotland) Ltd, Edinburgh, UK) and water *ad libitum*. Appropriate doses of indomethacin (Sigma; 10 mg/kg) or vehicle (4% gum acacia and 1% (v/v) TWEEN 20 surfactant, Sigma) or compound 1, were administered orally 2 hours before induction of oedema. A 0.1 ml volume of a 1% (w/v in 0.9% saline) λ-

carageenan (Sigma) was then injected into the right hind paw, while the contralateral hindpaws were injected with 0.1 ml saline to serve as a control. Oedema formation was measure hourly with calipers and results expressed as percentage of the maximum change in paw thickness.

Statistical analysis: All data points represent mean ± SEM values. Significance of difference with respect to control group was analysed by the two sample *t*-test.

Results

Compound 1 has been found to display anti-inflammatory activity *in vivo* as shown in Fig. 1 in which each point represents the mean ± SEM of 6-8 rats. Significantly different from the vehicle treated group, *p<0.05, **p<0.01, ***p<0.001. ■, vehicle; ▲, 1 10 mg/kg; ●, 1 50 mg/kg; ★, indomethacin 10 mg/kg. As may be seen from Fig. 1, injection of carrageenan into rat hind paw resulted in paw swelling, reaching a maximum increase of paw thickness by 50 ± 3 mm (n=8). Compound 1 showed a dose dependent inhibition of the carrageenan-induced oedema response in rats (Fig. 1). Significant anti-inflammatory activity was observed for compound 1 at a dose of 50 mg/kg and indomethacin (10 mg/kg) at all time points (Fig. 1).

The total inflammation during the five hour observation period was calculated from the area under the time course response curve. As expected vehicle treatment did not suppress the total inflammation (99 ± 3 of untreated group) while compound 1 at doses of 10 and 50 mg/kg significantly (p<0.05) reduced total inflammation to 85 ± 4 and 70 ± 5% respectively. While in the presence of the positive control, indomethacin (10mg/kg), the total inflammation was 57 ± 5 of control (untreated) group

Example 2B - In vitro Integrin-dependent Cell Adhesion inhibition by Compound 1

Endothelial cell-monocyte adhesion assay: Cell adhesion experiments were performed essentially as described previously (Ruoslahti, E. and Pierschbacher, M.D. (1987) Science 238, 491-497). Briefly, confluent monolayers of EAhy 926 endothelial cells were established in 96 well plates and activated by overnight incubation with human recombinant tumour necrosis factor-α (1 ng/ml; R & D Systems, Oxon, UK). [Methyl-^{3}H]thymidine (Amersham International plc, Little Chalfont, UK)-labeled and PMA (Sigma, Dorset, UK) activated monocytic U937 cells were then added to endothelial cells in the presence or absence of compound 1. Monocyte-endothelial cell adhesion was quantified as described by Habtemariam (Habtemariam, S. (1998) Planta Med. 64, 314-318).

Homotypic cell aggregation assay: The detailed assay protocol for PMA-mediated homotypic cell aggregation in U937 cells has been described (Li, R., Xie, J., Koistinen, V., Altieri, D.C., Nortamo, P. and Gahmberg, C.G. (1995) J. Cell Biol. 129, 1143-1153). Briefly, PMA (200 ng/ml) was added to cells in 96 well microtiter plates (2 x10^5 cells/well) in the presence or absence of compound 1. After four hours, cell aggregation was quantified and expressed as percentage of control (PMA alone) values (Li et al *ibid*).

Cell attachment assay: The adhesion of [Methyl-^{3}H]thymidine - labeled U937 cells to protein (fibronectin or ICAM-1) coated plates was measured as described previously (Ferreira, O.C., Valinsky, J.E., Sheridan, K., Wayner, E.A., Bianco, C. and Garcia-Pardo, A. (1991). Exp. Cell Res. 193, 20-26; Wilson, G.A. (1996). Cell adhesion molecules: fundamental facts. PP 24-32. R & D Systems, Abingdon, UK).

Results

The *in vitro* inhibitory effects of compound 1 on integrin-

mediated monocyte (U937 cell) adhesion to tumour necrosis factor-α(TNF)-activated EAhy endothelial cells, ICAM-1 or fibronectin coated plates and phorbol 12-myristate 13-acetate (PMA)-mediated homotypic cell aggregations are shown in Fig. 2 which shows the effects of compound **1** on U937 cell adhesion to EAhy 926 endothelial cells (■), ICAM-1 (●), and fibronectin (★), and homotypic cell aggregation of U937 cells (◆). Results are expressed as mean percentage of control values and SEM obtained from three separate experiments. The addition of compound **1** during the adhesion assay resulted in a concentration-dependent attenuation of monocyte adhesions with EC_{50} values between 7 and 20 μg/ml (Fig. 2). Pre-treatment of endothelial cells, ICAM-1 or fibronectin coated plates with compound **1**, however, did not result in suppression of monocyte adhesion (data not shown) suggesting that compound **1** acts through structural/functional interference with the integrin adhesion molecules on monocyte cell surface. As assessed by the MTT and thymidine incorporation assays (Habtemariam, S. (1997) Planta Med 63, 15-17), compound **1** did not affect the viability of cells at all concentrations tested (data not shown).

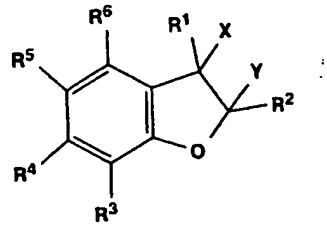
The leucocyte-specific β2 integrins (LFA-1, Mac-1 and CD11c/CD18) are involved in diverse cell adhesions required for leucocyte functions (Hynes, R.O. (1992) Cell 69, 11-25; Springer, T.A. (1995) Ann. Rev. Physiol. 57, 827-872; Bevilacqua, M.P., Nelson, R.M., Mannori, G., and Cecconi, O. (1994) Ann. Rev. Med. **45**, 361-378). Several studies using monoclonal antibodies have shown that the adhesion of PMA-activated U937 cells to TNF-activated endothelial cells and/or ICAM-1 is β2-integrin (LFA-1 and Mac-1) dependent (Habtemariam, S. (1998) Planta Med. 64, 314-318; Cavender, D.E., Edelbaum, D. and Welkovich, L. (1991) *J. Leukocyte Biol.*

49, 566-578). The PMA-mediated homotypic cell aggregation of U937 cells is also mediated through activation of a β2 integrin, LFA-1. It is now well established that the RGD sequence is the most common motif contained in several matrix proteins including fibronectin and serves as a recognition site for diverse integrin receptors (Ruoslahti, E. and Pierschbacher, M.D. (1987) Science 238, 491-497). The attachment of unstimulated U937 cells to fibronectin involves interaction of the RGD motif with two β1 integrins (α4β1 and α5β1) (Ferreira, O.C., *et al ibid* and references there in). The potent inhibitory activity of compound **1** towards both of the above β1 and β2 integrins-dependent cell adhesion indicates the relatively non-selective antagonism of integrins by compound **1** and implies that the compound has therapeutic potential for diseases where integrin adhesion molecules play a significant role.

CLAIMS

1. Use of a compound of general formula (I)

(I)



wherein each one of R^1 and R^2 is independently selected from optionally substituted and/or unsaturated C_1-C_8, alkyl and alkoxy, OH, and aryl, or represents ZCO_2 wherein Z is independently selected in each case from optionally substituted and/or unsaturated C_1-C_8, alkyl, and aryl, or R^1 has the same meaning as before, and R^2 is ZCO_2W wherein Z has the same meaning as before and W is CH_2CO or optionally substituted and/or unsaturated C_1-C_8, alkyl;
each one of R^3, R^4, R^5 and R^6 is independently selected from H, OH, Cl, Br, F, I, C_1-C_8, alkoxy, C_1-C_8, alkyl, R^7CO, NO_2, NR^8R^9 or ZCO_2 wherein 2 has the same meaning as before;
R^7 being H or C_1-C_8 alkyl, and
each one of R^8 and R^9 being independently selected from H and C_1-C_4 alkyl; and
each one of X and Y is H, one of X and Y is H and the other is OH, or X and Y together represent a double bond;
and physiologically acceptable salts thereof, in the preparation of a medicament for the treatment of an integrin mediated condition.

2. Use according to claim 1 of a compound of formula (I) wherein at least one of R^1 to R^6 is ZCO_2 wherein Z has the same meaning as before.

3. Use according to claim 2 of a compound of formula (I) wherein at least one of the R^1 and R^2 is ZCO_2 wherein Z has the same meaning as before.

4. Use according to any preceding claim of a compound of formula (I) wherein the alkyl or alkoxy groups comprising R^1 to R^9 or part thereof are C_1-C_6 alkyl or alkoxy.

5. Use according to any preceding claim of a compound of formula (I) wherein the alkyl groups comprising R^1 to R^9 are each independently selected from straight or branched chain alkyl each being independently selected from the group consisting of isopropyl, propyl, butyl, isobutyl, and tertbutyl; and the cyclic alkyl group cyclohexyl.

6. Use according to any previous claim wherein R^1 represents ZCO_2 wherein Z is optionally unsaturated C_1-C_6 alkyl, R^2 is selected from optionally substituted and/or unsaturated C_1-C_6 alkyl and aryl, and each one of R^3, R^4, R^5 and R^6 is independently selected from H, OH, Cl, Br, F, I, C_1-C_6 alkoxy, C_1-C_6 alkyl, R^7CO, NO_2 and NR^8R^9; R^7 being H or C_1-C_6 alkyl, and each one of R^8 and R^9 being independently selected from H and C_1-C_4 alkyl, and each one of X and Y is H, or X and Y together represent a double bond.

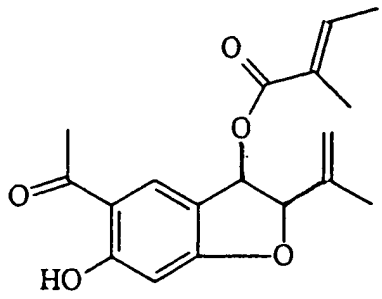
7. Use according to any preceding claim of a compound of formula (I) wherein R^2 represents optionally unsaturated C_1-C_4 alkyl.

8. Use according to any preceding claim of a compound of formula I wherein R^5 represents CH_3CO.

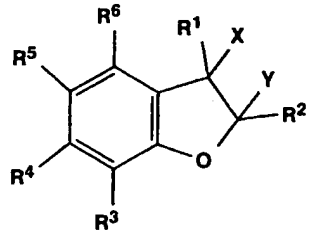
9. Use according to any preceding claim wherein R^4 represents OH and each of R^3 and R^6 represents H.

10. Use according to any preceding claim wherein R^1 is tiglinoyl ($CH_3CHC(CH_3)CO_2$).

11. Use according to claim 9 of the compound



12. A compound of formula (I)



wherein each one of R^1 and R^2 is independently selected from optionally substituted and/or unsaturated C_1-C_8, alkyl and alkoxy, OH, and aryl, or represents ZCO_2 wherein Z is independently selected in each case from optionally substituted and/or unsaturated C_1-C_8, alkyl, and aryl, or R^1 has the same meaning as before, and R^2 is ZCO_2W wherein Z has the same meaning as before and W is CH_2CO or optionally substituted and/or unsaturated C_1-C_8, alkyl;

each one of R^3, R^4, R^5 and R^6 is independently selected from H, OH, Cl, Br, F, I, C_1-C_8, alkoxy, C_1-C_8, alkyl, R^7CO, NO_2, NR^8R^9 or ZCO_2 wherein 2 has the same meaning as before;

R^7 being H or C_1-C_8 alkyl, and

each one of R^8 and R^9 being independently selected from H and C_1-C_4 alkyl; and

each one of X and Y is H, one of X and Y is H and the other is OH, or X and Y together represent a double bond;

and physiologically acceptable salts thereof in the preparation of a medicament for the treatment of an integrin mediated condition, with the proviso that when R^1 is $CH_3C{:}CHCH_3CO_2$, R^2 is $CH_3{:}CH_2$, R^4 is OH, and R^5 is $C{:}OCH_3$, then R^3 and R^6 are not both H.

13. A compound of formula (I) according to claim 12 wherein at least one of R^1 to R^6 is ZCO_2 wherein Z has the same meaning as before.

14. A compound of formula (I) according to claim 12 or claim 13 wherein at least one of the R^1 and R^2 is ZCO_2 wherein Z has the same meaning as before.

15. A compound of formula (I) according to any of claims 12 to 14 wherein the alkyl or alkoxy groups comprising R^1 to R^9 or part thereof are C_1-C_6 alkyl or alkoxy.

13. 16. A compound of formula (I) according to any of claims 12 to 15 wherein the alkyl groups comprising R^1 to R^9 are each independently selected from straight or branched chain alkyl each being independently selected from the group consisting of isopropyl, propyl, butyl, isobutyl, and tertbutyl; and the cyclic alkyl group cyclohexyl.

17. A compound of formula (I) according to any of claims 12 to 16 wherein Z is optionally unsaturated C_1-C_6 alkyl, R^2 is selected from optionally substituted and/or unsaturated C_1-C_6 alkyl and aryl, and each one of R^3, R^4, R^5 and R^6 is independently selected from H, OH, Cl, Br, F, I, C_1-C_6 alkoxy, C_1-C_6 alkyl, R^7CO, NO_2 and NR^8R^9; R^7 being H or C_1-C_6 alkyl, and each one of R^8 and R^9 being independently selected from H and C_1-C_4 alkyl, and each one of X and Y is H, or X and Y together represent a double bond.

18. A compound of formula (I) according to any of claims 12 to 17 wherein R^2 represents optionally unsaturated C_1-C_4 alkyl.

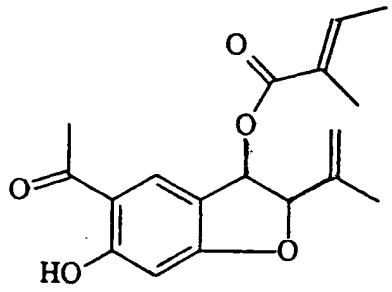
19. A compound of formula (I) according to any of claims 12 to 18 wherein R^5 represents CH_3CO.

20. A compound of formula (I) according to any of claims 12

to 19 wherein R^4 represents OH and each of R^3 and R^6 represents H.

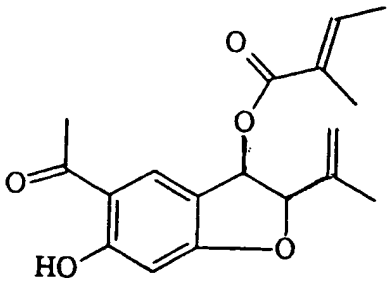
21. A compound of formula (I) according to any of claims 12 to 20 wherein R^1 is tiglinoyl ($CH_3CHC(CH_3)CO_2$).

22. A compound according to any of claims 12 to 21 which is



23. A pharmaceutical formulation comprising a compound of formula (I) as defined in claim 1 or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier therefor.

24. A pharmaceutical formulation comprising the compound

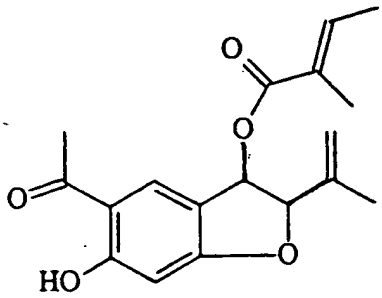


when isolated and/or purified from natural sources as described hereinbefore, or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier therefor.

25. A method for the treatment or prophylaxis of an integrin mediated condition in a manual, which method comprises the administration of a clinically useful amount of a compound of Formula (I) according to claim 1 or a pharmaceutically

acceptable salt or physiologically functional derivative thereof in a pharmaceutically useful form.

26. A method of treatment or prophylaxis of an integrin mediated condition in a mammal, which method comprises the administration of a clinically useful amount of the compound



or a pharmaceutically acceptable salt or physiologically functional derivative thereof in a pharmaceutically useful form.

27. A method according to claim 25 or claim 26 wherein said condition is selected from angina, inflammatory disease, asthma, cancer metastasis, coronary angioplasy, psoriasis, osteoporosis, thrombosis and viral and parasitic infections.

28. A method according to claim 27 wherein said inflammatory disease is selected from Arthritis; Atherosclerosis; Vasculitis; Ischemia and reperfusion injury; Adult respiratory distress syndrome; nephritis; Gastrointestinal inflammation (Inflammatory bowel disease)- Ulcerative colitis and Crohn's disease; Hepatic disease; CNS disease; Dermatosis; Graft rejection; Graft versus Host disease; and Sepsis.

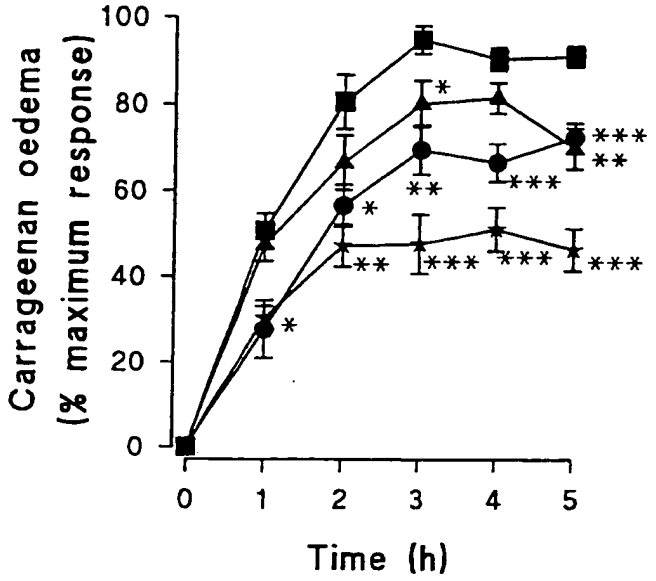


Fig. 1. *p<0.05, **p<0.01, ***p<0.001.
■, vehicle; ▲, 1 10 mg/kg; ●, 1 50 mg/kg; ★, indomethacin 10 mg/kg.

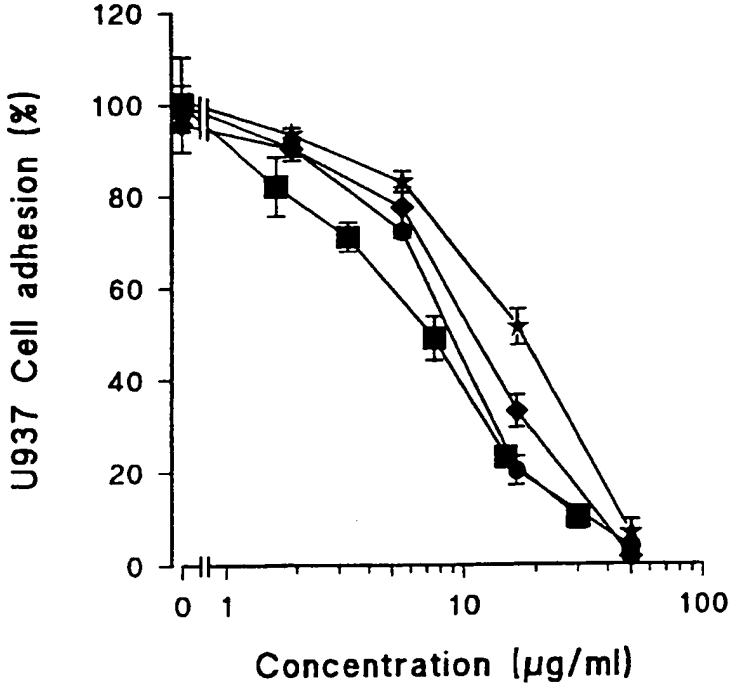


Fig. 2. EAhy 926 ■, ICAM-1 , fibronectin ★, U937 ◆